Possible immunologic involvement of antioxidants in cancer prevention[1,2]

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ABSTRACT The people of Linxian County, China have one of the world's highest rates of esophageal cancer. Two intervention trials were conducted to determine whether supplementation with specific vitamins and minerals could lower mortality from or incidence of cancer in this population and whether supplementation with multiple vitamins and minerals would reduce esophageal and gastric cardia cancer in persons with esophageal dysplasia. About 30 000 general population (GP) subjects in the GP trial were randomly assigned to one of eight intervention groups according to a one-half replicate of a 2^4 factorial experimental design and were supplemented for 5.25 y with four combinations of micronutrients at doses from one to two times the US recommended dietary allowance (RDA). About 3000 subjects in whom dysplasia was diagnosed in the dysplasia trial were randomly assigned to groups receiving daily supplementation with 14 vitamins and 12 minerals at two to three times the US RDA or placebo for 6 y. Results of the dysplasia trial indicate that in individuals with esophageal dysplasia, micronutrient supplementation had little effect on T lymphocyte responses. In contrast, male participants in the GP trial who were supplemented with β-carotene, vitamin E, and selenium showed significantly ($P < 0.05$) higher mitogenic responsiveness of T lymphocytes in vitro than those not receiving these micronutrients. *Am J Clin Nutr* 1995;62(suppl):1477S–82S.

KEY WORDS Antioxidants, micronutrients, β-carotene, vitamin E, selenium, immune responses, T lymphocytes, whole-blood cultures, cancer mortality

INTRODUCTION

Cancer of the upper gastrointestinal tract is common in China; a nationwide retrospective cancer mortality survey during the 1970s revealed geographical clustering of cancer of the esophagus and gastric cardia. The people of Linxian County have one of the world's highest rates of esophageal cancer (1). This clustering provided a unique opportunity to conduct epidemiologic, etiologic, and prevention studies of human cancer. Two intervention trials were conducted in Linxian County, China to determine whether supplementation with specific vitamins and minerals could lower the incidence of or mortality from cancer in this population at high risk (2–5). One trial was conducted in the general population (GP trial), involving 29 584 apparently healthy individuals, 40–69 y of age at the start of supplementation and without previous history of cancer. Subjects in the GP trial were randomly assigned to one of eight intervention groups to receive different combinations of micronutrients according to a one-half replicate of a 2^4 factorial experimental design. As shown in **Table 1** subjects were supplemented for 5.25 y with four combinations of micronutrients or placebo at doses of one to two times the US recommended dietary allowances (RDAs) (6): A, retinol and zinc; B, riboflavin and niacin; C, vitamin C and molybdenum; and D, β-carotene, vitamin E, and selenium (2). A second trial was conducted in a population of 3318 persons with cytologic evidence of esophageal dysplasia (dysplasia trial) who were randomly assigned to receive daily supplementation with 14 vitamins and 12 minerals at doses of two to three times the US RDAs or placebo for 6 y (4) (**Table 2**). Cancer incidence and mortality were registered during both trials (2–5).

At the end of the trials, all participants received a physical checkup and a subgroup of participants had venous blood collected for nutrient and immunologic lymphocyte analyses. In subgroups of ≈400 subjects from each trial, cellular immune function tests that could be done in a laboratory in the Linxian field station were conducted. The nutritional and immunology objectives of the GP and dysplasia trials were to ascertain whether micronutrient intervention could play a role in the prevention and/or development of esophageal cancer via the cellular immune system.

SUBJECTS AND METHODS

At the end of intervention in March and April 1991, 400 participants from the GP trial and 375 from the dysplasia trial were randomly selected and their cellular immune responses studied.

Collection of blood

Ten milliliters blood from fasted persons was collected from the antecubital vein by use of all-plastic sterile syringes

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TABLE 1
Types and daily doses of micronutrients by treatment supplement[1]

Supplement and micronutrients	Dose per day
A	
Retinol (as palmitate; IU)	5000
Zinc (as zinc oxide; mg)	22.5
B	
Riboflavin (mg)	3.2
Niacin (mg)	40.0
C	
Ascorbic acid (mg)	120
Molybdenum (as molybdenum yeast complex; μg)	30
D	
β-Carotene (mg)	15
Selenium (as selenium yeast; μg)	50
α-Tocopherol (mg)	30

[1] Described in detail in reference 2.

(Sarstedt Inc, Newton, NC) to which 1000 U sodium heparin (Sigma Chemical Company, St Louis) had been added under sterile conditions. After blood was collected from subjects in the villages, it was transported by vehicle for 0.5–1.5 h at ambient temperature to the field station laboratory.

Preparation of whole-blood cultures for T lymphocyte proliferation ex vivo

On arrival of blood at the laboratory, 170 μL blood was removed under sterile conditions and placed in a polystyrene tube (Falcon; Becton Dickinson Labware, Lincoln Park, NJ) in preparation for dilution for T lymphocyte proliferation cultures. The aliquot was diluted to a final volume of 680 μL with RPMI-1640 cell culture medium (Gibco Laboratories, Grand Island, NY) containing 2.0 mmol L-glutamine and penicillin-streptomycin/L at 100 000 U/L and 100 mg/L, respectively; referred to hereon as RPMI-1640.

Preparation of peripheral blood mononuclear cells

After removal of the blood aliquot for whole-blood cultures the remaining blood was centrifuged at 1200 × *g* for 15 min. The plasma was withdrawn and an equal volume of phosphate-buffered saline (PBS) was added. After it was mixed, the blood cell suspension was overlaid on a ficoll-hypaque density (1.074) gradient and centrifuged at 450 × *g* for 30 min. The mononuclear cells at the interface were collected and washed three time with PBS; each wash was followed by 10-min centrifugations at 1000 × *g*. The number of viable cells was counted in a hemocytometer by using the trypan blue exclusion technique. The cells were suspended in RPMI-1640 and the cell concentration readjusted to 2×10^9 cells/L.

Stimulants of T lymphocytes

Stock solutions of concanavalin-A (ConA, Type IV, no. C-2010, Sigma Chemical Company) and phytohemagglutinin-M (PHA, no. L-2646, Sigma Chemical Company) were prepared at concentrations of 2 g/L in RPMI-1640, divided into aliquots, and stored at −20 °C. Monoclonal antibody to CD3 was kindly provided as a gift by YX Chen (Cancer Institute, Chinese Academy of Medical Sciences, Beijing, People's Republic of China). Newly thawed aliquots of each stimulant were prepared each day.

TABLE 2
Daily doses and types of micronutrients in the supplements for the dysplasia trial[1]

Vitamin or mineral and compound	Dose
β-Carotene (mg)	15
Vitamin A (acetate; IU)	10 000
Vitamin E (2-*ambo*-α-tocopherol; IU)	60
Vitamin C (ascorbic acid; mg)	180
Folic acid (μg)	800
Thiamin (thiamin mononitrate; mg)	5
Riboflavin (mg)	5.2
Niacinamide (mg)	40
Vitamin B-6 (pyridoxine HCl; mg)	6
Vitamin B-12 (cyanocobalamin; μg)	18
Vitamin D (IU)	800
Biotin (μg)	90
Pantothenic acid (calcium pantothenate; mg)	20
Calcium (dibasic calcium phosphate; mg)	324
Phosphorus (dibasic calcium phosphate; mg)	250
Iodine (potassium iodide; μg)	300
Iron (ferrous fumarate; mg)	54
Magnesium (magnesium oxide; mg)	200
Copper (cupric oxide; mg)	6
Manganese (manganese sulfate; mg)	15
Potassium (potassium chloride; mg)	15.4
Chloride (potassium chloride; mg)	14
Chromium (chromium chloride; μg)	30
Molybdenum (sodium molybdate; μg)	30
Selenium (sodium selenate; μg)	50
Zinc (zinc sulfate; mg)	45

[1] Participants received two multivitamin, multimineral tablets (Centrum; Lederle Laboratories Inc, Wayne, NJ) and one β-carotene capsule (Solatene; Hoffmann-LaRoche Inc, Nutley, NJ) or matching placebos daily. Supplement described in detail in reference 4.

Lymphocyte proliferation assays

In vitro whole-blood cell cultures received, in order, 50 μL diluted (1:4) whole blood (final dilution at 1:16 per culture), 50 μL RPMI-1640 alone (background) or RPMI-1640 containing designated stimulant (PHA, ConA, or antibody to CD3), and 100 μL RPMI-1640 per well of round-bottom, 96-well microtiter plates (Corning Glass Works, Corning, NY). In vitro mononuclear cell cultures received, in order, 50 μL adjusted peripheral blood mononuclear cells (PBMNCs; 1×10^5 cells/culture), 50 μL RPMI-1640 alone (background) or RPMI-1640 containing designated stimulant (PHA, ConA, or antibody to CD-3), and 100 μL RPMI-1640 containing 20% (10% final concentration) heat-inactivated (56 °C for 30 min) certified fetal bovine serum (Gibco) per well of flat-bottom, 96-well microtiter plates (Falcon; Becton Dickinson Labware).

Proliferative activity of T lymphocytes was based on the incorporation of tritiated thymidine ([methyl-^{3}H], specific activity of 888 GBq (24 Ci)/mmol; Chinese Atomic Energy Institute, Beijing) in DNA synthesis by cells in triplicate cultures for background and each stimulant in both types of cell culture. PHA and ConA were added at a concentration of 1.56 μg/culture (7.8 mg/L) and antibody to CD3 at a concentration of 10.5 μg/culture (52.5 mg/L). Cell cultures were incubated for 84 h at 37 °C in 5% carbon dioxide and 95% humidity. Thirteen hours before the end of incubation, 37 μBq (1.0 μCi) [^{3}H]thymidine was added to each culture. After incubation the

cells were harvested with a Cell Harvester (Skatron Inc, Sterling, VA). The [^{3}H]thymidine-labeled DNA was collected on 12-well filtermats (Skatron). The filtermats were air-dried, wrapped in wrap-film, and shipped to the central laboratory for scintillation counting. Individual filter discs with scintillant (Ready Safe; Beckman Instruments Inc, Fullerton, CA) were counted in a Beckman LS-3801 scintillation counter with a single-label DPM (decays per minute) program. Proliferative activity is expressed as corrected Bq ($\bar{x} \pm$ SE); background was subtracted from stimulated. Note that the value in Bq times 60 equals 1.0 DPM.

T lymphocyte responsiveness to interleukin 2

To each well of a 24-well plate, 1×10^6 PBMNCs in 1 mL complete medium (CM, equal to RPMI-1640 with 15% newborn calf serum) supplemented with 10 μg PHA were added and the cells incubated at 37 °C in 5% carbon dioxide and 95% humidity for 96 h. The cells were then washed three times with PBS, counted by use of the trypan blue exclusion technique, and resuspended in CM. Two hundred microliters of the PBMNC suspension containing 1.5×10^4 cells with or without 1 U recombinant human interleukin 2 (rhIL-2, Cetus) was transferred to flat-bottom, 96-well microtiter plates and incubated at 37 °C in 5% carbon dioxide and 95% humidity for 72 h. Sixteen hours before termination of culture, [^{3}H]thymidine [18.5 μBq (0.5 μCi)/well] was added to each culture and the radioactive incorporation was measured as described above. The results are expressed as a stimulation index (SI), ie, the ratio of mean [^{3}H]thymidine incorporation in triplicate cultures of rhIL-2 that were stimulated to that in the unstimulated control.

Natural killer and lymphokine-activated killer cell activity

The target cell used for the natural killer (NK) cell activity assay was K562; that used for the lymphokine-activated killer (LAK) cell activity assay was Raji. Cells in culture at logarithmic phase were harvested, suspended in CM ($1–2 \times 10^9$ cells/L), and labeled with 370 μBq (10 μCi) [^{3}H]thymidine at 37 °C in an incubator in 5% carbon dioxide for 2 h. After they were thoroughly washed with PBS, the cells were resuspended in CM at a concentration of 2×10^9 cells/L. For the LAK cell activity assay, 1×10^6 PBMNCs in 1 mL CM were cultured in a 24-well plate in the presence of 50 U rhIL-2 for 96 h. The rhIL-2-activated cells were collected, washed, counted, and resuspended in CM at a concentration of 5×10^9 cells/L. For the NK cell activity assay, untreated PBMNCs were suspended in CM at the same cell concentration.

The cytolytic assay was performed as follows. To each well of a flat-bottom, 96-well PBMNC microtiter plate was added 1×10^4 K562 cells in 50 μL CM and 5×10^5 untreated PBMNCs in 100 μL CM (for the NK cell assay), or 1×10^4 Raji cells in 50 μL CM and 5×10^5 rhIL-2-treated PBMNCs in 100 μL CM (for the LAK cell assay) at an effector-to-target cell ratio of 50:1. The cultures were incubated at 37 °C for 18 h. Before cell harvest, 0.6 mg trypsin (Difco Laboratories, Detroit) and 10 μg DNAse (Sigma) in 50 μL PBS were added to each well with slight agitation and incubation was continued at 37 °C for 30 min. The percentage of specific lysis of target cells was calculated from the radioactivity (counts per minute, or CPM) of labeled target cells that survived, according to the following equation:

$$\text{Specific lysis (\%)} = 1 - \left(\frac{\text{Mean CPM of survived target cells}}{\text{mean CPM of target cells}}\right) \times 100\% \quad (1)$$

where the target cells are referred to as those cultured in the absence of effector cells.

TABLE 3
Proliferative responsiveness of T lymphocytes in whole-blood cultures to mitogens in males in the general population trial[1]

Intervention group	PHA	ConA	anti-CD3
		Bq	
Placebo (n = 30)	1388 ± 111	636 ± 64	286 ± 86
AB (n = 25)	1565 ± 141	754 ± 75	199 ± 70
AC (n = 24)	1243 ± 137	617 ± 80	90 ± 33
AD (n = 23)	1950 ± 195[2]	859 ± 112	367 ± 125
BC (n = 29)	1171 ± 152[2]	630 ± 107	151 ± 48
BD (n = 19)	1597 ± 128	636 ± 83	146 ± 54
CD (n = 20)	1334 ± 160	630 ± 75	193 ± 72
ABCD (n = 29)	1430 ± 143	636 ± 70	182 ± 60

[1] $\bar{x} \pm$ SE. PHA, phytohemagglutinin-M; ConA, concanavalin A; anti-CD3, antibody to CD3. Micronutrient supplements A, B, C, and D are described in Table 1.
[2] Significantly different from each other, $P = 0.05$, Tukey's studentized range test multiple-comparison procedure.

Statistical methods

Comparisons of GP treatment group means used an F statistic for a test of overall equality and Tukey's multiple-comparison method for testing the equality of all pairs of treatment groups (7). Individual effects of the four treatments were estimated by a linear regression model,

$$E(Y_i) = \alpha_0 + \alpha_A A_i + \alpha_B B_i + \alpha_C C_i + \alpha_D D_i + \beta\, age_i \quad (2)$$

where, for the i^{th} subject, Y_i denotes the immunology measure being analyzed, A_i through D_i = 1 (or 0) if the subject was given (or not given) treatments A, B, C, or D, accordingly, and

TABLE 4
Proliferative responsiveness of T lymphocytes in whole-blood cultures to mitogens in females in the general population trial[1]

Intervention group	PHA	ConA	anti-CD3
		Bq	
Placebo (n = 18)	1148 ± 184	511 ± 92	180 ± 85
AB (n = 20)	1662 ± 166	587 ± 88	155 ± 71
AC (n = 19)	1294 ± 194	630 ± 107	115 ± 53
AD (n = 22)	1402 ± 182	505 ± 81	162 ± 55
BC (n = 27)	1534 ± 138	593 ± 83	192 ± 69
BD (n = 18)	1581 ± 126	593 ± 83	135 ± 57
CD (n = 13)	1581 ± 126	453 ± 63	158 ± 90
ABCD (n = 18)	1148 ± 184	511 ± 92	180 ± 85

[1] $\bar{x} \pm$ SE. PHA, phytohemagglutinin-M; ConA, concanavalin A; anti-CD3, antibody to CD3. Micronutrient supplements A, B, C, and D are described in Table 1.

TABLE 5
Proliferative responsiveness of T lymphocytes, measured using whole-blood cultures and estimated from regression model, in males in the general population trial[1]

Supplement	PHA		ConA		anti-CD3	
	Estimated effect	*P*	Estimated effect	*P*	Estimated effect	*P*
	Bq		*Bq*		*Bq*	
A	166	0.10	65	0.26	35	0.61
B	−53	0.60	−35	0.55	−79	0.24
C	−269	0.01	−63	0.29	−169	0.01
D	198	0.05	11	0.85	45	0.51

[1] Micronutrient supplements A, B, C, and D are described in Table 1. Statistical analysis according to reference 7.

age_i denotes the subject's age at the start of the study. Therefore, subjects in the population group receiving both A and C would have $A_i = C_i = 1$ and $B_i = D_i = 0$. The effects of treatment interactions were also estimated by the regression model but were not statistically significant and therefore are not discussed. Comparisons of treated and control groups in the dysplasia trial used a *t* statistic. The SAS procedure GLM was used for the calculations (8).

RESULTS

GP trial

Proliferative responsiveness of T lymphocytes in whole-blood cultures to PHA, ConA, and antibody to CD3 from either males or females in the GP trial who received different combinations of supplements A, B, C, D, or placebo are presented in **Table 3** and **Table 4**, respectively. Males in the GP trial who received supplement A (retinol and zinc) plus D (β-carotene, vitamin E, and selenium) showed the highest proliferative responsiveness of T lymphocytes in whole-blood cultures to PHA. Males who were supplemented with B (riboflavin and niacin) plus C (vitamin C and molybdenum) showed the lowest response. This difference between groups AD and BC was statistically significant ($P = 0.05$; Table 3). No other pair of treated groups was significantly different from one another and no treated group was significantly different from the group that received placebo. No differences were observed among any of the groups when the cultures were stimulated with Con-A and antibody to CD3, nor were any differences observed in cultures from females stimulated with each of the three mitogens (Table 4).

Changes in proliferative responsiveness of T lymphocytes in whole-blood cultures to PHA, ConA, and antibody to CD3 in males in the GP trial according to vitamin-mineral supplement are shown in **Table 5**. Males who received supplement D (β-carotene, vitamin E, and selenium), compared with those who did not, showed significantly higher proliferative responsiveness of T lymphocytes to PHA ex vivo. In contrast, males who received supplement C (vitamin C and molybdenum), compared with those who did not, showed significantly lower proliferative responsiveness of T lymphocytes to PHA and antibody to CD3 ex vivo.

LAK cell activities of PBMNCs from males and females in the GP trial who received different combinations of supplements A, B, C, D, or placebo are shown in **Table 6**. Males who received the total combination of supplements (A, B, C, and D) showed significantly less LAK cell activity than did those supplemented with A (retinol and zinc) plus B (riboflavin and niacin) or A plus D (β-carotene, vitamin E, and selenium). Although males in the AD group showed the highest mean LAK cell activity, it was not significantly different from activity in the placebo group. There were no differences among females in the different supplement groups in LAK cell activity.

TABLE 6
Lymphokine-activated killer cell activity in the general population trial[1]

Intervention group	Specific lysis	
	Males	Females
	%	
Placebo	37.1 ± 12.5 [7]	33.8 ± 19.1 [6]
AB	54.3 ± 6.9[a] [6]	27.0 ± 16.1 [4]
AC	35.6 ± 18.3 [5]	43.7 ± 21.5 [10]
AD	61.5 ± 5.3[a] [4]	24.8 ± 20.0 [5]
BC	34.4 ± 19.0 [5]	20.7 ± 9.7 [4]
BD	40.7 ± 15.9 [7]	50.5 ± 7.7 [2]
CD	49.0 ± 21.8 [3]	40.0 ± 43.8 [2]
ABCD	28.4 ± 16.5[b] [10]	39.0 ± 32.7 [6]

[1] $\bar{x}$ ± SD; *n* in brackets. Micronutrient supplements A, B, C, and D are described in Table 1. Mean values marked with different superscript letters are significantly different, $P < 0.05$ (Tukey's studentized range tests multiple-comparison procedure).

Dysplasia trial

The proliferative responsiveness of T lymphocytes as measured by the whole-blood assay or by the conventional technique of using separated cell cultures to PHA, Con-A, and antibody to CD3 is presented in **Table 7**. The correlation in mitogenic responsiveness of T lymphocytes in these two forms of cell culture is presented in **Table 8**. Subjects supplemented with multiple vitamins and minerals showed no significant differences from the placebo group in proliferative responsiveness of T lymphocytes in whole-blood or PBMNC cultures to the three mitogens. We found a highly significant correlation in mitogenic proliferation of T lymphocytes between whole-blood assays and PBMNC cultures by testing samples from 374 subjects in the dysplasia trial. The highest correlation ($r = 0.69$) between the results through use of the two techniques was found in cultures stimulated with PHA.

Resting T lymphocytes do not express the α chain (Tαc) of the IL-2 receptor. Under mitogenic stimulation, with Tαc expression T lymphocytes become responsive to IL-2 stimulation. The results in **Table 9** indicate that T lymphocyte respon-

TABLE 7
Comparison of proliferative responsiveness of T lymphocytes measured using whole-blood and separated cell cultures from subjects in the dysplasia trial[1]

	Whole-blood cultures		Separated cell cultures	
Mitogen	Placebo (n = 187)	Supplemented (n = 187)[2]	Placebo (n = 181)	Supplemented (n = 185)
		Bq		
PHA	1762 ± 58	1730 ± 64	1791 ± 75	1815 ± 80
ConA	788 ± 31	770 ± 36	1526 ± 76	1581 ± 79
anti-CD3	172 ± 23	166 ± 23	469 ± 48	521 ± 49

[1] $\bar{x}$ ± SE. PHA, phytohemagglutinin-M; ConA, concanavalin A; anti-CD3, antibody to CD3.
[2] Supplement described in Table 2.

TABLE 8
Correlations for lymphocyte proliferation values from both whole-blood and separated cell cultures stimulated with phytohemagglutinin (PHA), concanavalin A (ConA), and antibody to CD3 (anti-CD3)[1]

Mitogen	r	P
PHA	0.69	<0.0001
ConA	0.49	<0.0001
anti-CD3	0.46	<0.0001

[1] n = 374.

siveness to rhIL-2 in PBMNC cultures was not different between the supplemented and placebo groups for males and females. As summarized in **Table 10**, the NK and LAK cell activities of PBMNCs from the supplemented and placebo groups for both series were not significantly different.

DISCUSSION

The GP and dysplasia trials offered a unique opportunity to examine the relation among micronutrients, immunity, and cancer in a population at high risk of cancer. As reported by Blot et al (*see* Table 3 in reference 3) at this conference, 32% of all deaths during the 5.25-y GP trial were caused by cancer of the esophagus or stomach.

Among males in the GP trial, those who received supplement D showed significantly higher T lymphocyte responsiveness to PHA compared with those who did not. Other studies have shown that supplementation of humans with antioxidants such as β-carotene, vitamin E, and selenium separately or in combination can cause immunoenhancement (9–13). Diets that caused low vitamin E (11) and β-carotene (13) status in adult males and females, respectively, suppressed T lymphocyte responsiveness in vitro. van Poppel et al (9) reported that male smokers (mean age ≈40 y) supplemented for 14 wk with 20 mg β-carotene/d showed increased mitogenic responsiveness of PBMNCs cultured in their own plasma and stimulated with PHA. Fuller et al (10) reported that supplementation with 30 mg β-carotene/d protected against photosuppression of in vivo delayed-type hypersensitivity (DTH) of cell-mediated immunity to recall bacterial antigens in males (aged 19–39 y) consuming a low-carotene diet for 66 d.

Using the dietary approach of micronutrient insufficiency, Kramer et al (13) showed reduced T lymphocyte proliferation ex vivo to PHA in women fed a low-carotene, controlled diet. This reduced proliferation was reversed after supplementation with a carotenoid-rich concentrate from foods. Meydani et al (14) reported that supplementation of healthy elderly subjects with 800 IU 2-*ambo*-α-tocopherol for 30 d significantly improved their DTH response, mitogenic response of T lymphocytes to ConA, and ConA-stimulated IL-2 production. Kramer et al (11) also reported the immunoenhancing effects of vitamin E in middle-aged males with immunosuppression induced by n−3 fatty acids. Immunoenhancement has also been shown in elderly humans supplemented with 100 μg selenium/d for 6 mo (12).

The limited information on the role of antioxidants and immunity in humans is supported by findings from animal models. Bendich (15) reviewed the enhancing effects of β-carotene on T lymphocyte responses and tumoricidal capacities of cytotoxic T lymphocytes, NK cells, and macrophages. Jyonuchi et al (16) reported an augmenting effect of β-carotene and carotenoids without provitamin A activity on antibody production in response to T-dependent antigen. Tomita et al (17) reported an augmentation of tumor immunity against syngeneic tumors in mice by β-carotene in vivo. One of our laboratories (YH Zhang) reported immunopotentiating effects of vitamin A and selenium in mice. Retinoic acid and its analogues were

TABLE 9
T cell responsiveness to interleukin 2 in the dysplasia trial[1]

	Stimulation index	
Sex	Supplemented	Placebo
Male	3.7 ± 3.6 [87]	3.2 ± 2.9 [103]
Female	3.9 ± 4.1 [100]	3.4 ± 2.5 [80]

[1] $\bar{x}$ ± SD; n in brackets. Supplement described in Table 2.

TABLE 10
Natural killer (NK) and lymphokine-activated killer (LAK) cell activity in the dysplasia trial

	Specific lysis	
Sex and intervention group[1]	NK	LAK
	%	
Male		
Supplemented (n = 87)	43.2 ± 16.9	37.1 ± 15.5
Placebo (n = 103)	40.0 ± 16.4	39.4 ± 16.9
Female		
Supplemented (n = 100)	38.5 ± 16.2	38.9 ± 17.1
Placebo (n = 80)	38.9 ± 15.4	41.2 ± 21.2

[1] $\bar{x}$ ± SD. Supplement described in Table 2.

found to significantly enhance specific cytotoxic T lymphocyte induction against allogeneic and syngeneic tumor cells (18, 19). Zhang (20) also reported that selenium could augment cell-mediated immunity in normal mice, and that a proper dose of selenium supplement could completely restore NK cell activity and cytotoxic T lymphocyte response against tumor growth in selenium-deficient mice (21).

Presented immunologic results from the GP and dysplasia trials provide information that is helpful for understanding the relation between micronutrient nutriture, immunity, and cancer. The major difference in immune results between the GP and dysplasia trials was the effect of certain micronutrient combinations on T lymphocyte proliferation responses in the high-risk GP trial population compared with the dysplasia trial subjects with precancerous lesions. Immune results of the dysplasia trial indicate that in persons with esophageal dysplasia, micronutrient supplementation had little effect on T lymphocyte responses. In contrast, participants in the GP trial experienced a beneficial effect of antioxidant supplementation with β-carotene, vitamin E, and selenium on T lymphocyte responses ex vivo. The suppressive effects of supplement C (vitamin C and molybdenum) on mitogenic responsiveness of T lymphocytes to PHA and antibody to CD3 ex vivo (Table 5) in males is unclear. More work is needed to clarify this finding.

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